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ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITY OF *UTICA DIOICA* LEAVES ON STZ INDUCED TYPE 1 DIABETIC MODEL RATS

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ABSTRACT

In the present study we have explored the effect of *Urtica dioica*, a traditional antidiabetic herb used in India and Bangladesh, on insulinemic and chronic inflammatory status of type 1 diabetic model rats. The mature and fresh leaves of *U. dioica* was collected from the mountain range of Assam in India and the water extract of the leaves was used at a dose of 1.25g/kg body weight. Adult Long-Evans male rats, bred at BIRDEM Animal house, were used in this study. Type 1 DM was produced with single intraperitoneal injection of Streptozotocin using standardized methods. The experiment for semi-chronic effects was done with continuous feeding of water extract of *U. dioica* for 8 days. Serum glucose estimated by glucose oxidase (GOD-POD) method, serum insulin was measured by ELISA and CRP (as a marker for chronic inflammation) was also measured by an ELISA technique specific for rats. Serum lipids were measured by enzymatic-colorimetric methods. When compared to Control the water extract of *U. dioica* showed a significant hypoglycemic effect on 8th day (Fasting blood glucose, mmol/L, M±SD, 25.19±6.4 in Control group vs 8.8±3.7 in *U. dioica* group, $p<0.001$). The reduction of serum glucose level was accompanied by a substantial (about 300%) rise of serum insulin levels on 8th day (Fasting serum insulin, µg/ml, M±SD, 0.315±0.269 in Control vs 0.105±0.086 in *U. dioica* groups on 0 day, p=ns; 0.284±0.208 in Control vs 0.417±0.361 in *U. dioica* groups on 8th day, p=0.004). The CRP also showed lower values, but the difference was not significant compared to Control (Fasting CRP, µg/ml, 275±78 in Control group vs 271±68 *U. dioica* group on 0 day; 232±85 in control vs 307±72 on 8th day in *U. dioica* group). Triglycerides level decreased significantly in *U. dioica* treated group when compared with the Control group (Fasting TG level, mg/dl, M±SD, 119±28 in the Control Vs 37±11 *U. dioica* group, $p<0.001$). *U. dioica* water extract has hypoglycemic properties, which may have an association with improved insulinemic status linked to an anti-inflammatory effect of the plant product on pancreatic β-cells.

INTRODUCTION: Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and it is mainly linked with low blood insulin level or insensitivity of target organs to insulin. It is the most prevalent chronic disease in the world affecting nearly 25% of the population [1]. Traditional preparations of plant sources are widely used almost everywhere in the world to treat this disease. Therefore, plant materials are considered to be the alternative sources for finding out new leads for hypo-/antihyperglycemic agents.

Urtica dioica (Family-Urticaceae), nettle a kind of herb, found in the South Asian region has been identified as a potential anti-diabetic plant. It has been found that, there is induction of insulin secretion by a component of *Urtica dioica* leave extract in perfuses islets of langerhans and its in vivo effects in normal and streptozotocin induced rat [2]. The blood glucose lowering effect of *Urtica dioica* (Stinging Nettle) as a medicinal plant has been noted in old writings such as those of Avicenna. Recently, there have also been other investigators that indicated the hypoglycemic effect of *Urtica dioica*. Even though in many research work it have been established that *Urtica dioica* has hypoglycemic effect, but on the contrary in some of the research done by various scientist, suggest that it does not contain any hypoglycemic effect. In fact the chronic effect of the hydro-alcoholic extract of *Urtica dioica* leaves has no hypoglycemic effect [3]. Some studies also suggest that it have Antihyperglycemic activity as aqueous extract [4].

As there is contradictory view regarding the effect as hypoglycemic/antihyperglycemic, so it is difficult to establish its actual mode of action on diabetes. As the mechanism of this effect has not been deduced, therefore, we decided to investigate the anti-diabetic and anti-inflammatory activity of our plant nettle leaves on non-diabetic and diabetic models rats.

MATERIALS AND METHODS:

Plant materials and preparation of test sample: The mature leaves of *Urtica dioica* was collected from the mountain range of Assam in India. Leaves were washed with water and then air dried and oven dried at 40°C temperature. The dried leaves were then grinded to make powder, which were then sieved to get fine powder. The powders were then soaked in distilled water to get suspension of solution. These suspensions were filtered with thin and clean cloth and then filtered by filter paper. The suspensions were evaporated by BUCHI Rota vapor R-114 [BUCHI, Germany], connected with BUCHI water bath B-480 at 50°C. In this case, 72mbar (to remove water) pressure and 160rpm rotation speed were maintained constantly. Finally, small amount of liquid were evaporated from the semi-solid extracts by using a freeze-drier (HETOSICC, Heto Lab Equipment, Denmark).

Experimental Animals: The study was conducted with adult Long-Evans rats of both sexes (weighing 180-220g). They were bred at the BIRDEM animal house maintained at a constant room temperature of 22 ± 5^0C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure when animals were used after 12hrs fasting. The rats had no access to food during the whole period of blood sampling. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

Induction of Type 1 Diabetes to the Rats: Type 1 diabetes was induced by a single intraperitoneal (*i.p.*) injection of streptozotocin (STZ, Upjohn Company, Kalamazoo, MI USA) at a dose of 65 mg/kg body weight (dissolved in 0.1 citrate buffer, pH 4.5) to adult rats (3-4 months). Confirmatory fasting blood glucose test for type 1 model rats was performed after 7 days of STZ injection. Rats with fasting blood glucose of 18 mmol/l or above were considered as Type 1 [5]. The animals were divided into 3 groups (normal control, Type 1 controls, *U. dioica* treated) of 5-8 rats in each group. Rats of all groups were kept under similar environmental conditions, and were provided with enough food and water throughout the experiment.

Semi-chronic Study: The experiment was done for 8 days. Normal control and Type 1 control rats were fed with deionized water at a dose of 10ml/kg body weight, and test group were fed with *U. dioica* extract at a dose of 1.25gm/kg body weight/10ml of water [6]. Blood samples were collected by amputation of the tail tip under mild diethyl ether anesthesia [7]. Blood samples were collected on the 0 day and 8th day to measure serum glucose, total cholesterol, triglyceride, insulin and C-reactive protein (CRP). On 8th day of experiment, the rats were decapitated. The body weight of each rat was measured in the 0 or 1st, 3rd, 6th and 8th day of experiment.

Biochemical analysis: Serum glucose was measured by glucose-oxidase method (Sera Pak, USA). The total cholesterol and triglyceride (TG) were measured by enzymatic-colorimetric method (Randox Laboratories Ltd., UK). Serum insulin by Rat Insulin enzyme linked immunosorbent assay (ELISA) method (Crystal Chem Inc., USA) and Serum CRP was measured by C-reactive protein assay (Helica Biosystem, Inc., USA). The absorbance was measured by microplate ELISA Reader (Bio-Tek EL-340, USA).

Statistical Analysis: Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 12 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD as appropriate. Statistical analysis of the results were performed by using the student's t-test (paired) or ANOVA (analysis of variance) followed by Bonferroni post hoc test. The limit of significance was set at $p<0.05$.

RESULTS:

Semi-chronic effect on fasting glucose level: It was found from that fasting serum glucose levels on 0 day were higher in Type 1 control and *U. dioica* treated rats in comparison to normal rats. After 8 days treatment, fasting blood glucose level of type 1 control and extract treated group decreased significantly ($p=0.000$), when comparisons were done between normal control-Type 1 control and Type 1 control-extract treated group. Here the water extract of *U. dioica* showed a significant hypoglycemic effect (**Table 1**).

TABLE 1: SEMI-CHRONIC EFFECT OF *U. DIOICA* WATER EXTRACT ON FASTING GLUCOSE LEVEL OF TYPE 1 DIABETIC MODEL RATS

Group	Glu_0day (mMol/l)	Glu_8th day (mMol/l)
Normal Control (n = 5)	7.8±1.6	6.8±1.2
Type 1 control (n =8)	28.4±6.5	25.19±6.4*a
U. dioica treated (n =5)	21.52±2.5	8.8 ±3.7*b

Data are presented as Mean±SD. a=significant change when compared with normal control with Type 1 control; b= significant change when compared with Type 1 control with *U. dioica* treated group. *p=0.000

Effect on the body weight (BW): It was found that there was a gradual increase in body weight of normal control groups of rats on 8 day; Type1 control rats showed an increase of 1% on 8th day. Where as the body weight was decreased in the *U. dioica* treated group and reduction of body weight were 7% at 3rd day, 15% at 6th day and 21% at 8th day when compared with the initial value. Statistically significant results were observed when comparisons were done between normal control-Type 1 control ($p<0.01$; $p<0.001$) and Type 1 control-extract treated group ($p<0.01$; $p<0.001$ and $p=0.000$) (**Table 2**).

TABLE NO. 2: SEMI-CHRONIC EFFECT OF *U. DIOICA* WATER EXTRACTS ON BODY WEIGHT OF TYPE 1 DIABETIC MODEL RATS

Group	BW_ 1st day (gm)	BW_ 3h day (gm)	BW_ 6h day (gm)	BW_ 8 day (gm)
Normal Control (n = 5)	214±27	216±28	224±29	230±32
Type 1 control (n =8)	173±22	173±23 a *	175±23 a **	175±23 a **
U. dioica treated (n =5)	186±10	173±14 b*	158±11***	148±18***

Data are presented as Mean±SD and compared using ANOVA (Bonferroni test); a = significant change when compared with normal control with Type 1 control; b= significant change when compared with Type 1 control with *U. dioica* treated group; * p< 0.02-0.01; ** p<0.006-0.001; ***p=0.000

Effects on lipidemic status: *U. dioica* has some effect on atherogenic lipids. It was found that there were no significant changes in the total cholesterol and serum triglycerides level among the normal groups, but total cholesterol and serum triglycerides were decreased in the normal control rats when compared with the 0 day value (56±5 vs. 55±4 and 65±19 vs. 49±7 respectively). It was also found that, there were no significant changes of total cholesterol in the type 1 control and *U. dioica* water extract treated group on 8th day. But the changes of cholesterol level were 68±15 vs. 76±31 and 58±11 vs. 42±2 respectively. Whereas the triglyceride levels were significantly high (p=0.000) in type 1 control group when compared with normal control (49±7 vs. 119±28) and significantly low (p=0.000) in *U. dioica* extract group when compared with type 1 control group (119±28 vs. 37±11) (**Table 3**)**.**

TABLE 3: SEMI-CHRONIC EFFECT OF *U. DIOICA* WATER EXTRACT ON LIPIDEMIC STATUS OF TYPE 1 DIABETIC MODEL RATS

Group	CH_0 (mg/dl)	CH_8 (mg/dl)	TG_0 (mg/dl)	TG_8 (mg/dl)
Normal Control (n = 5)	56±5	55±4	65±19	49±7
Type 1 control (n =8)	68±15	76±31	113±29	119±28*a
U. dioica treated (n =5)	58±11	42±2	91±15	37±11*b

Data are presented as Mean±SD and compared using ANOVA (Bonferroni test); a= significant change when compared with normal control with Type 1 control; b= significant change when compared with Type 1 control with *U. dioica* treated group; * p=0.000

Effect on serum insulin level: It was found that insulin level was increased significantly (p=0.004) in the extract treated group (0.776±0.282 vs 0.417±0.361) and significantly low (p=0.04) in the type1 control group (0.776±0.282 vs 0.284±0.208) when compared with the normal control on 8th day. It may indicate that 8 days treatment with this *U. dioica* water extract may exert insulin secreting activity by stimulating the residual pancreatic β-cells of Type1 diabetic model rats (**Fig. 1**).

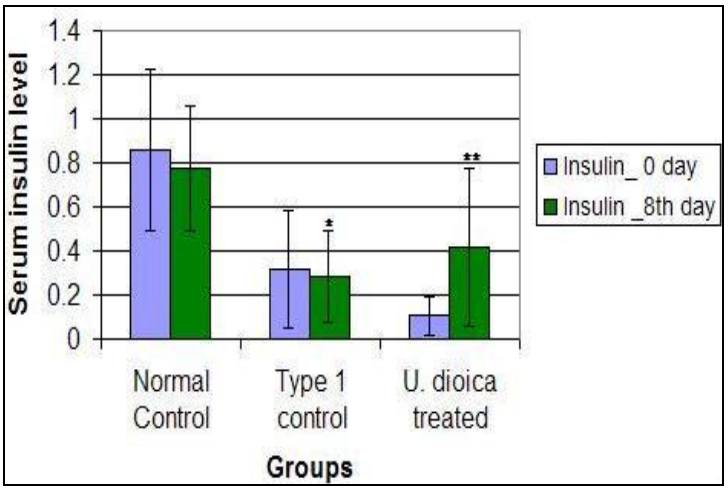

FIG. 1: SEMI-CHRONIC EFFECTS OF *U. DIOICA* WATER EXTRACT ON SERUM INSULIN LEVEL OF TYPE1 DIABETIC MODEL RATS

Effect on serum CRP level: It was found that the fasting value of serum CRP level was increased in the normal control group on 8 days in comparison to initial day but it was within the normal range (Ref value <300 µg/ml). Fasting CRP value was also increased in type 1 control group after 8 days. It was also found that fasting serum CRP level was reduced on 8th day in comparison to 0 day in the extract treated group (271±68 vs 232±85). CRP level was lower in *U. dioica* treated groups (232±85) in comparison to type 1 control (307±72) after 8 days administration of *U. dioica*, but the difference was not significant. This result suggests that 8-days treatment with *U. dioica* water extract can reduce the inflammatory biomarker in Type1 diabetic model rats and thereby improving the inflammatory condition in type 1 diabetes (**Table 4**).

TABLE NO. 4: SEMI-CHRONIC EFFECT OF *U. DIOICA* WATER EXTRACT ON SERUM CRP LEVEL OF TYPE 1 DIABETIC MODEL RATS AFTER 8 DAYS OF FEEDING

Group	Serum CRP (µg/ml)	
	Day 0	Day 8
Normal Control (n = 5)	224±112	293±69
Type 1 control (n =8)	275±78	307±72
U. dioica treated (n =5)	271±68	232±85

Data are presented as Mean±SD and compared using ANOVA (Bonferroni test)

DISCUSSION: The rapidly increasing prevalence of diabetes throughout the world will continue to challenge the scientists and clinicians in refining existing therapies and developing new approaches to counter this devastating disease. The scope for the discovery and development of new anti-diabetic therapies from nature/plants is vast and merits corresponding attention. Although oral hypoglycemic agents and insulin is the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have well known side effects and fail to significantly alter the course of diabetic complications [8].

As the knowledge of heterogeneity of this disorder increases, it is needed to look for more efficacious agents with lesser side effects. Moreover, the existing drugs do not modify the course of diabetic complications. In relation to plants also, barring a few studies [9, 10, 11, 12, 13] most of the studies have not assumed the impact of these plants on the course of diabetes and its complications, particularly the macro-vascular pathologies. Since inflammation plays an important role in the pathogenesis of diabetes so exploration of new anti-inflammatory compounds from the nature is also an important task as various synthetic anti-inflammatory drugs produce solemn adverse effects in different vital organs in the body.

Our present study was undertaken to assess the hypoglycemic properties (semi-chronic effects) with underlying mechanism of action of *U. dioica* and also to evaluate anti-inflammatory effect with particular focus on serum CRP level. In the present investigation, it was found that in type 1 diabetic model rats STZ produced significant increase in fasting glucose levels ranging between 20.94±5.69 to 25.11±2.08 mmol/l. Injection of STZ produces fragmentation of DNA of pancreatic β-cells, which stimulates poly (ADP-ribose) and depletes NAD. Ultimately it leads to destruction of β-cells and it is evidenced by hyperglycemia and hypoinsulinemia [14].

In the present study, the experimental type 1 diabetic model rats were hyperglycemic and hypoinsulinemic. *U. dioica* was fed to type1 diabetic model for 8 days consecutive days. Treatment with *U. dioica* significantly reduced the serum glucose levels (p=0.000; fasting blood glucose, mmol/l, M±SD, 25.19±6.4 in the control group Vs 8.8±3.7 in *U. dioica* group). Dyslipidemia is an important risk factor for atherosclerotic complications of diabetes [15]. Hypercholesterolemia and hypertriglyceridaemia have been reported to occur in STZ induced diabetic rats [16]. Apart from blood glucose lowering activity of *Urtica dioica*, changes in lipid profile have also been observed. Regarding total cholesterol level, no significant reduction was found. However, favorable effect on triglycerides was observed. There was a 39% reduction of cholesterol level when compared with type1 control and 26% reduction compared with normal control rats at the end of the experimental period.

There was a substantial increase (almost 300%) in serum insulin level of *U. dioica* treated group in comparison to type1 control rats (Fasting serum insulin µg/ml, M±SD, 0.315±0.269 in control Vs 0.105±0.086 in *U. dioica* treated group on 0 day p=Ns; 0.284±0.208 in control Vs 0.417±0.361 in *U. dioica* treated group on 8th day, p=0.004). In the context of the pathophysiology of STZ-induced type 1 diabetic rats, the improvement in insulin levels in response to the *Urtica dioica* water extract seemed to be mediated through improvement in β-cell morphology and/or function. Prevention of ongoing β-cell damage, recovery of partially damaged β-cells, regeneration of new cells and stimulation of insulin secretion in functional cells is among the alternate possibilities induced by the water extract.

STZ is itself an inflammatory drug, which can produce increased level of C-reactive protein (CRP) [17]. CRP levels are elevated in type 1 diabetes [18] have reported that elevated CRP levels may provide an additional marker for risk of progression to type 1

diabetes. In the present study, CRP level of STZ induced type 1 rats were found to be decreased after 8 days of treatment with *Urtica dioica* (Fasting CRP, µg/ml 275±78 in type1 control Vs 271±68 in *U. dioica* group on 0 day; 307±72 in control Vs 232±85 in *U. dioica* treated group). Therefore, it may be suggested that *Urtica dioica* may work as an anti-inflammatory agent and thereby improving the glycemic status in type 1 diabetic rats. The anti-inflammatory effect, in turn may be associated with the histological and functional improvement of β-cells with the consequence of improved insulinemic status.

CONCLUSION: Thus it can be concluded that, *Urtica dioica* has got promising hypoglycemic and hypolipidemic effects in respect to Type 1 diabetic model rats and these effects are mediated by the central effect on the histological and/or functional status of pancreatic β-cells with a resultant increase in insulin secretion. The improved β-cell histology and/or function induced by *Urtica dioica* in type 1 diabetic rats are likely to be associated with an anti-inflammatory activity.

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